Biosorption of zinc ions from aqueous solution by the microalga *Scenedesmus obliquus*

Cristina M. Monteiro · Paula M. L. Castro · F. Xavier Malcata

Abstract Aquatic environments are often exposed to toxic heavy metals, which gain access to the food chain via microalgae and may cause severe problems at higher trophic levels. However, such a metabolic specificity can be taken advantage of in bioremediation strategies. The potential of a novel wild strain of *Scenedesmus obliquus*, previously isolated from a heavy metal-contaminated site in northern Portugal, to remove Zn from aqueous solutions was thus studied, using several initial concentrations. The removal extent reached its maximum by 1 day: 836.5 mg Zn/g biomass, at the initial concentration of 75 mg/L, mainly by adsorption onto the cell surface. Comparative studies encompassing a commercially available strain of the same microalgal species led to a maximum removal extent of only 429.6 mg Zn/g biomass, under identical conditions. Heat-inactivated cells permitted a maximum removal of 209.6 mg Zn/g biomass, at an initial concentration of 50 mg Zn/L. The maximum adsorption capacity of Zn, estimated via Langmuir's isotherm, was 330 mg Zn/g biomass. Finally, Zn removal was highest at pH 6.0–7.0. It was proven, for the first time, that such a wild microalga can uptake and adsorb Zn very efficiently, which unfolds a particularly good potential for bioremediation. Its performance is far better than similar (reference) species, especially near neutrality, and even following heat-treatment.

Keywords Microalgae · Heavy metal removal · Bioremediation · Inactivated cells · pH · Langmuir isotherm

C. M. Monteiro · P. M. L. Castro · F. Xavier Malcata (✉)
CBQF/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal
e-mail: fxmalcata@esb.ucp.pt

Introduction

Contamination of natural water streams by toxic metal cations arises because of several industrial activities (Aksu and Dönmez 2006). Such a form of pollution constitutes a major environmental concern, because those contaminants are extremely toxic to the human being, as well as to the flora and fauna of the effluent water-receiving bodies, even at minor concentrations (Incharoensakdi and Kitjaharn 2002). This happens owing to their tendency to (bio) accumulate throughout the food chain (Doshi et al. 2007), since they are not biodegradable. Classical technologies applied to remove toxic metals from aqueous solutions, viz. ion exchange and salt precipitation, are often inefficient or too expensive when heavy metals are present at very low concentrations (Incharoensakdi and Kitjaharn 2002; Gupta and Rastogi 2008). Therefore, it is important to devise efficient biochemical methods to remove those toxic elements, with the further requirement of being environment friendly.

Sorption of heavy metals, using microorganisms as biosorbents, offers a potential alternative to conventional processing methods, mainly because of their low cost, strong metal binding capacity, high efficiency in dilute effluents and environment friendliness (Philips et al. 2003; Gupta and Rastogi 2008). Biosorption takes advantage of the ability of biological materials to accumulate toxic metals from surrounding aqueous solutions (e.g. wastewater), via both metabolically mediated and physico-chemical pathways (Radway et al. 2001; Gupta and Rastogi 2008). Microalgae have proven their high metal-binding capacities (Schiewer and Volesky 2000; Bayramoğlu et al. 2006; Muñoz et al. 2006), even when compared to such other biological entities as bacteria, fungi and yeasts (Tüzün et al. 2005), owing to the presence of polysaccharides, proteins

and/or lipids on the surface of their cell walls: these contain charged functional groups (e.g. amino, hydroxyl, carboxyl and sulphate) that can attract, and thus act as binding sites for metals (Bayramoğlu et al. 2006; Gupta and Rastogi 2008). Bioremoval of metal ions using microalgal cells is affected by several factors, including the specific surface properties of the microorganism and the biomass concentration, as well as physicochemical parameters of the solution, such as pH and initial metal ion concentration (Aksu and Dönmez 2006). Both viable and inactivated microbial cells can be used to remove toxic metals from solution (Dönmez et al. 1999; Abu Al-Rub et al. 2004), although it has been described that inactivated cells are more profitable for industrial applications, as they are not affected by the toxicity of said metals (Chu and Hashim 2004).

The extensive use of Zn cations in a few industrial applications, e.g. for galvanization and electroplating (Ahuja et al. 1999; Senthilkumar et al. 2006), without proper downstream recovery, has led to contamination of soil and fresh water habitats (Vasconcelos and Tavares 1998; Oliveira et al. 2001). Despite this metal being an essential element for activation of some enzymes in human cells, high exposure levels (100–500 mg/day) can be toxic and even carcinogenic (Omar 2002; Senthilkumar et al. 2006).

In the present study, a freshwater microalga strain of *Scenedesmus obliquus* was isolated from the surrounding area of a large industrial complex of essentially chemical facilities, which have released for decades their wastewaters into a nearby stream—"Esteiro de Estarreja". Among the metals present in those sediments, Zn appears as one of the main contaminants, with concentrations up to 3,620 mg/kg (Oliveira et al. 2001).

The major goal of this research effort was thus to test the ability of the isolated *S. obliquus* cells (hereafter denoted as strain L—for Local isolate) to remove Zn ions from solution, both in viable and inactive forms, when exposed to various initial Zn concentrations. Bioremoval under a range of initial pH values was also tested. Additionally, the removal capacity of a commercial ecotype of the same species *S. obliquus* (hereafter denoted as ACOI 598) was also assessed, in order to compare the efficiency of the isolated wild strain to that of a commercial counterpart.

Materials and methods

Microalga culture conditions

Cells of the freshwater green microalga *S. obliquus* (L) were isolated from a heavy metal-polluted site in northern Portugal, and of *S. obliquus* (ACOI 598) were obtained from Coimbra Culture Collection of Algae (ACOI), held by the University of Coimbra (Portugal).

Both strains of *S. obliquus* were cultivated in PHM medium (Borowitzka and Borowitzka 1988), containing 1 g/L Tris–HCl buffer but without ethylenediaminetetracetic acid (EDTA). Cultures were maintained at 25°C, under continuous light, at an irradiance of 29.18 μE/(s.m^2), provided by cool light fluorescent lamps. In all experiments, the microalgal cells were harvested at the exponential phase (after 2–3 days of growth), and then used as inoculum.

Cell growth was determined by measuring absorbance at 600 nm in a Shimadzu mini 1240 spectrophotometer (Japan), and subsequently converting it to dry weight (DW) via a previously prepared calibration curve (which had been validated for various stages of microalgal growth).

A stock solution of Zn ions was prepared by dissolving $ZnCl_2$ in deionised water, to a final concentration of 5 g/L of Zn^{2+}. For each experiment, an appropriate volume of the stock solution was then added to the culture medium, in order to obtain the desired final supernatant concentration.

All material used to handle and grow the microalgae was previously rinsed with nitric acid, and then several times with deionised water, so as to prevent interferences with the analytical assays.

Biomass growth and metal removal by living cells

In order to study the Zn removal capacity by the microalgal cells, batch tests of both strains were performed in triplicate using an initial viable biomass dose of 0.02 g/L, exposed to Zn concentrations of 0, 10, 25, 50 and 75 mg/L. Duplicate samples of 75 mL were collected at the beginning of the experiment, and then daily for a period of 7 days, and used to quantify biomass and Zn removal (both via adsorption on, and via intracellular accumulation in microalgal cells). Metal removal from solution was assayed as follows: samples were centrifuged at 4,000 rpm for 15 min at 4°C, and the supernatant was collected to measure the residual amount of dissolved metal. Afterwards, the pellet was washed for 20 min with an aqueous solution of 0.02 M EDTA (to remove Zn ions adsorbed onto the cell surface), thereby allowing only intracellular Zn to be determined. The washing EDTA fraction was discarded, and cells were again centrifuged. The final pellet was digested with 1 mL of 15 M HNO_3 and 0.5 mL of 70% $HClO_4$, to release and thus allow determination of the amount of intracellular metal, according to the method proposed by Matsunaga et al. (1999) and Pérez-Rama et al. (2002). Zn concentration in samples of the supernatant solution was determined using atomic absorption spectrophotometry, with air-acetylene flame atomization (FA-AAS) and background correction, in a Perkin Elmer 3100 (USA) spectrophotometer using a wavelength of 213.9 nm. The total Zn removed by weight of microalgal cells was calculated as the difference between the initial

and the remaining Zn concentration in the supernatant; the amount of Zn adsorbed onto the cell surface was determined as the difference between the total Zn removed and its intracellular fraction. A blank control, containing culture medium plus metal, was also established for each concentration, and showed that the Zn concentration remained stable in those flasks for the time period of each experiment. The initial Zn concentrations in experimental flasks were confirmed by taking a 5 mL-sample of culture medium containing metal (and before adding the microalga culture), and assaying by FA-AAS as described previously.

Metal removal by inactivated cells

For this specific part of the study, *S. obliquus* (L) cells were previously inactivated by heating at 100°C for 24 h. The metal removal capacity of inactivated cells was then determined by exposing an initial microalgal dose of 0.02 g/L to initial Zn concentrations of 10, 25, 50 and 75 mg/L, in batch tests performed in triplicate, in a water bath stirred at 100 rpm and kept at 25°C. Samples were collected at 0, 5, 15, 30, 60 and 90 min, and then centrifuged at 6,000 rpm for 15 min at 4°C. The total metal removed (considered here as the total amount adsorbed) was evaluated as described previously for the viable biomass. The amount of metal removed by 90 min was considered to be that associated with thermodynamic equilibrium conditions.

Effect of pH on metal removal

To ascertain how pH affects metal removal, triplicate batch tests were performed by exposing living cells, at an initial biomass dose of 0.35 g/L, to a concentration of 50 mg/L Zn, at pH values ranging from 3 to 7; pH was measured (using a Crison Micro pH 2001) only at the beginning and at the end of the experiment, since previous experience indicated that pH did not undergo any significant variation within the experiment timeframe. Samples were collected at pre-determined time intervals (0, 5, 15, 30, 45, 60, 90 and 120 min), and analysed, as described previously, for the residual Zn concentration in solution, in order to estimate the total extent of Zn removal.

Statistical analyses

The statistical analyses performed took advantage of the SPSS software, v. 16.0 (SPSS, Chicago IL, USA); the experimental data were subject to analysis of variance (ANOVA). To detect the statistical significance of differences between means (at the 5% level), Student's *t*-test and Tukey's test were further applied. The parameters of the Langmuir isotherm were fitted to the equilibrium data via nonlinear regression analysis.

Results and discussion

Biomass growth and metal removal by living cells

The Zn removal capacity of *S. obliquus* (strains L and ACOI 598) biomass was investigated at different initial metal concentrations, for a period of 7 days, and cell growth was also followed; the corresponding biomass growth pattern obtained for ecotype L is shown in Fig. 1. Growth inhibition was higher at higher Zn concentrations; the same occurred for strain *S. obliquus* (ACOI 598), for which a significant growth inhibition over time was observed as Zn concentration increased (data not shown). Based on ANOVA, one confirmed that an increasing initial Zn concentration reduced significantly ($P < 0.05$) microalgal growth. Such a growth inhibition, brought about by increasing initial metal concentration, has also been reported elsewhere (Omar 2002; Pérez-Rama et al. 2002; Rangsayatorn et al. 2002).

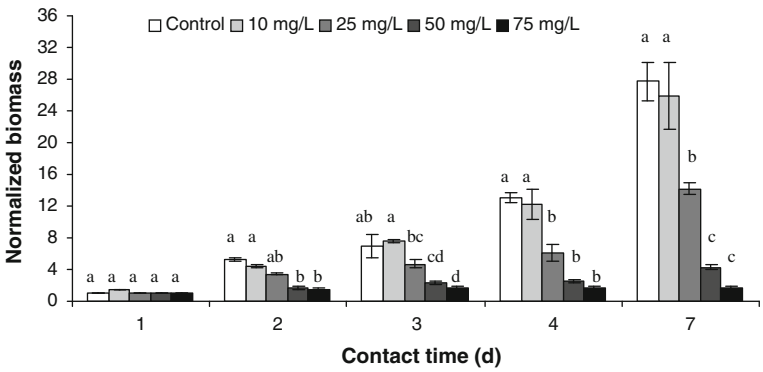

Fig. 1 Relative growth of *Scenedesmus obliquus* (L), at several initial Zn concentrations, throughout incubation time. Results are expressed as means; *error bars* represent standard deviations ($n = 3$). Means for the same time of collection, labelled with different letters, are significantly different from each other ($P < 0.05$). The biomass concentration is normalized by its initial dose

The total Zn removed, both by adsorption and via intracellular accumulation, by the two strains of *S. obliquus* was examined for a period of 7 days, upon exposure to various initial Zn concentrations (10, 25, 50 and 75 mg/L); the results produced are shown in Fig. 2. The metal removal was higher at higher initial metal concentrations. The strains of *S. obliquus* attained a Zn removal extent of 71.3 and 35.6 mg Zn/g, for the (L) and (ACOI 598) ecotypes, respectively, at the lowest initial metal concentration tested (10 mg Zn/L); when exposed to 75 mg Zn/L (the highest concentration), maximum removal extents were reached, viz. 836.5 and 429.6 mg Zn/g, respectively. The highest Zn mass removal occurred in cultures exposed to the highest metal concentration, despite the realisation that very little growth took place in these cultures. On the other hand, in cultures that reached a higher cell density, the amount of Zn removed per unit biomass decreased as the amount of biomass increased.

Additionally, living cells of both species exhibited an initial fast Zn removal, with the highest value achieved by 1 day, except for the (L) ecotype exposed to 10 mg/L, which reached the maximum removal only by 2 days. After this fast increase in metal sorption from solution, a decrease in the total concentration of Zn removal was detected until the last day of the experiment. Furthermore, it could be observed that, for both microalga strains and at

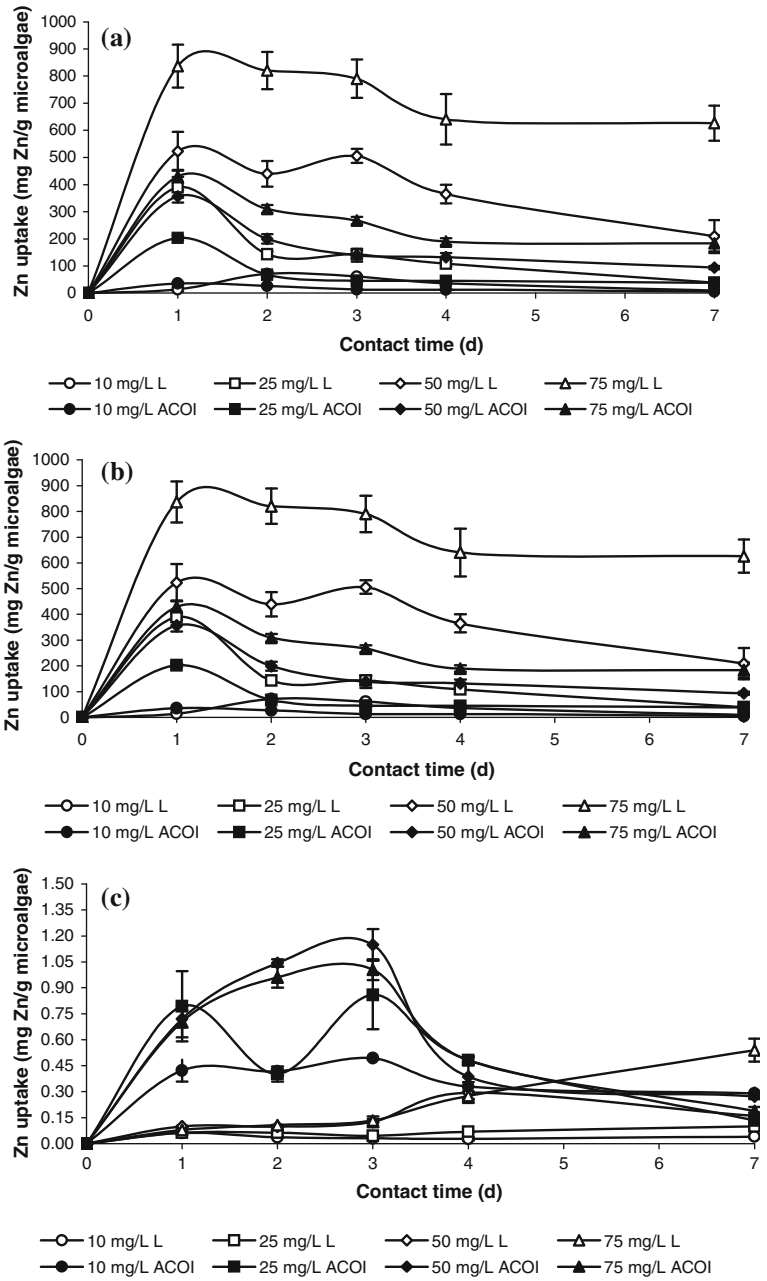

Fig. 2 Total amount of Zn removed (**a**), adsorbed (**b**) and accumulated intracellularly (**c**), per unit biomass, at several initial Zn concentrations (10, 25, 50 and 75 mg/L), by *Scenedesmus obliquus* (L) (*open symbols*) and *S. obliquus* (ACOI 598) (*solid symbols*), throughout incubation time. Results are expressed as means; *error bars* represent standard deviations ($n = 3$)

all concentrations tested, the (total) Zn removal was attained mainly by adsorption of metal ions onto the surface of the microalgal cells; note that intracellularly accumulated metal (Fig. 2c) was very low, viz. 0.54 and 1.15 mg/g at most, when in contact with a solution at an initial Zn concentration of 75 and 50 mg/L, by *S. obliquus* (L) and (ACOI), respectively. It has been described that cell wall is the first barrier to uptake of metals, and that surface adsorption is an important mechanism of defence that eventually permits microalgae to tolerate high levels of toxic metals in their surrounding medium (Lombardi et al. 2002).

Comparison via Student's *t*-test of the removal capacity of *S. obliquus* (L) with that of its commercial ecotype indicates that the wild strain removed significantly ($P < 0.05$) more metal than *S. obliquus* (ACOI 598), except at 10 mg/L by 1 day, when the (ACOI 598) ecotype removed significantly ($P < 0.05$) more, and at 25 mg/L by 7 days, when no significant ($P > 0.05$) differences were observed. The aforementioned higher Zn removal by *S. obliquus* (L) biomass may be attributed to the fact that these cells have been isolated from a polluted environment, and have thus developed, over sequential generations, a competitive advantage owing to environmental pressure. It has been described elsewhere that cells isolated from polluted environments demonstrate indeed a unique resistance to toxic pollutants, and a high binding affinity thereto (Nishikawa and Tominaga 2001; Tam et al. 2001). Likewise, in a previous study (Monteiro et al. 2009), a strain of the microalga *Desmodesmus pleiomorphus*, isolated from a polluted environment as well, exhibited higher metal removal capacity than its corresponding strain from a culture collection.

The highest capacity of metal removal found in the present study (836.5 mg Zn/g biomass) is comparable to data reported in the literature encompassing *Oscillatoria anguistissima* and *Microcystis* sp. (641.25 and 999.50 mg Zn/g biomass, respectively), by Ahuja et al. (1999) and Pradhan et al. (1998), respectively. However, our values obtained for Zn removal by either strain of *S. obliquus* are much higher than those reported by Incharoensakdi and Kitjaharn (2002) and Vannela and Verma (2006) for *Aphanothece halophytica* and *Spirulina platensis*, viz. 133 and 250 mg Zn/g, respectively.

In general, one found that *S. obliquus* (L) biomass yielded higher removal efficiency than its (ACOI 598) counterpart, with most metal being removed by adsorption onto the cell surface.

Metal removal by inactivated cells

Zinc biosorption by heat-inactivated *S. obliquus* (L) cells was investigated at several initial Zn concentrations (10, 25, 50 and 75 mg/L), using 90 min as contact time; the results are shown in Fig. 3. After addition of the inactivated biomass, the concentration of Zn^{2+} in solution dropped rapidly within the time interval 5–15 min, with an apparent equilibrium being established after 30 min. Ahuja et al. (1999) and Özer et al. (2000) observed a similarly rapid physical adsorption within the initial 15 min of contact, and maintenance of that level over the time remaining. This fast disappearance of Zn ions in solution suggests that the metal removal by thermally inactivated cells occurs exclusively by adsorption onto the microalga cell surface, i.e. independently of its rate of metabolism. This assumption is supported by the observations of Kaduková and Virčíková (2005), pertaining to the presence of copper on the cell surface of *Chlorella kessleri*, using transmission electron microscopy. Furthermore, Veglio and Beolchini (1997) hypothesized that physical adsorption of the metal via binding to the functional groups of cell wall polysaccharides is the most likely mechanism accounting for copper uptake by the microalga *Chlorella vulgaris*.

Analysis of Fig. 3 reveals that the loading capacities varied from 19.0 (at 10 mg/L) to 209.6 mg Zn/g (at 50 mg Zn/L). According to the results of ANOVA of the

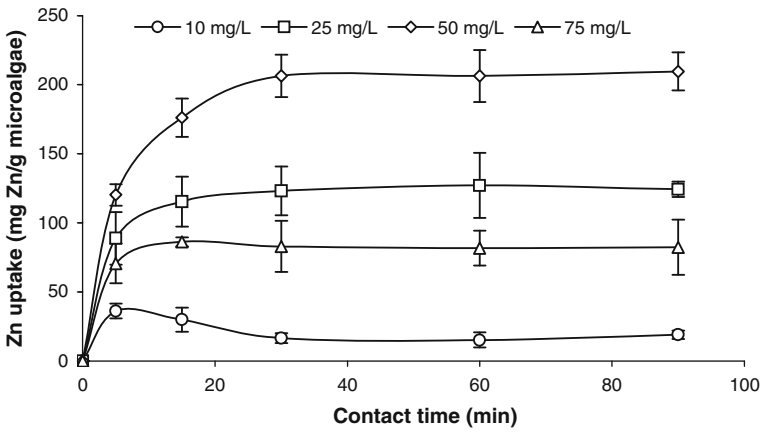

Fig. 3 Total amount of Zn removed, per unit biomass, by inactivated *Scenedesmus obliquus* (L), at several initial Zn concentrations (10, 25, 50 and 75 mg/L), throughout incubation time. Results are expressed as means; *error bars* represent standard deviations ($n = 3$)

experimental data pertaining to Zn removal vs. initial Zn concentration in solution, the total Zn removed by 90 min was significantly ($P < 0.05$) different at all concentrations tested, reaching its maximum value at 50 mg Zn/L. Considering that the total uptake of Zn by inactivated cells from the supernatant solution occurs exclusively via adsorption, and comparing the total amount of Zn adsorbed by both inactivated (at 90 min) and living cells (by 1 day) using a Student's *t*-test, it can be concluded that heat-inactivated biomass removes significantly ($P < 0.05$) less metal than living cells: this is so at all Zn concentrations tested but the lowest one, for which no significant ($P > 0.05$) differences could be found. Such an observation is probably associated to a partial disruption of structural components in the cell walls owing to the heating/drying process, which leads to protein denaturation, and is thus responsible for the decrease in number of the functional sites available to interact with Zn ions (as occurs in living cells).

Adsorption isotherms have been commonly used to describe experimental results encompassing uptake of metal ions by microorganisms. Data on Zn uptake by 90 min (taken operationally as the time to reach a state of thermodynamic equilibrium) are plotted in Fig. 4, overlaid on the adsorption isotherm following the Langmuir model; the best parameter estimates were $q_{max} = 330$ mg/g and $b = 0.03$, corresponding to the following equation:

$$Q_{eq} = \frac{q_{max} b C_{eq}}{1 + b C_{eq}},$$

where Q_{eq} denotes the amount of Zn adsorbed per unit weight of biomass at equilibrium (mg Zn/g biomass), C_{eq} is the residual supernatant metal concentration at equilibrium (mg Zn/L solution), and q_{max} and b are the two adjustable parameters, which can be viewed as the maximum (theoretical) adsorption capability of the biomass and the affinity thereof to the metal, respectively. A typical adsorption behaviour results—and a lack of fit analysis indicates that the aforementioned Langmuir model suffices to fit the experimental data, given their intrinsic spread and number of degrees of freedom available. The estimate obtained for q_{max} (i.e. 330 mg Zn/g biomass) is much higher than those reported by Omar (2002) and Vannela and Verma (2006), who obtained q_{max} values of 6.67, 5.03 and 44.24 mg/g for *S. obliquus*, *S. quadricauda* and *Spirulina platensis* (oven-dried), respectively, when in the presence of Zn. On the other hand, parameter b (which is related to the affinity between sorbent and sorbate) showed a low value ($b = 0.03$) when compared with those found by Omar (2002)—0.19 and 0.27 for *S. obliquus* and *S. quadricauda*, respectively, but a higher affinity than that reported by Vannela and Verma (2006)—0.0005 for *Spirulina platensis* (oven-dried). Therefore, the magnitude of our estimate of b suggests a good affinity of Zn for the inactivated biomass as sorbent material. On the other hand, the saturation concentration conveyed by q_{max} indicates a good removal capacity of the heavy metal under scrutiny, although the saturation concentration was not attained under the conditions used. Therefore, inactivated *S. obliquus* (L) appears to be an attractive biosorption material.

Our experimental results thus indicate that heat-inactivated microalgal cells are a good adsorbing matrix for toxic metals, although to a lesser degree than living cells. This form of biomass may consequently be a promising material for bioremediation of wastewaters contaminated with toxic metals, since it does not require nutrients for survival.

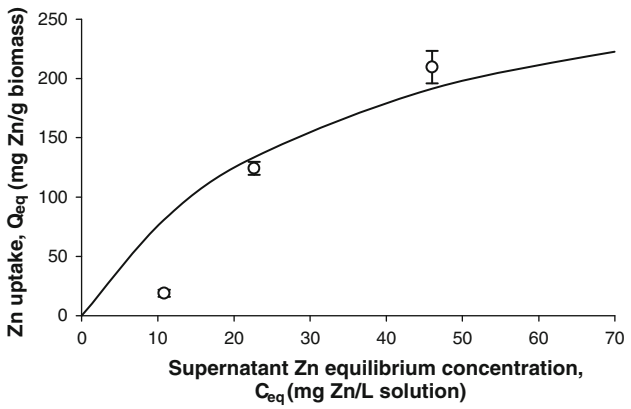

Fig. 4 Total amount of Zn removed, by inactivated *Scenedesmus obliquus* (L) (*open symbols*), at several final equilibrium Zn concentrations of the supernatant solution, overlaid with the best fit Langmuir isotherm (*solid line*). Results are expressed as means; *error bars* represent standard deviations ($n = 3$)

Effect of pH on metal removal

Many studies have shown that the pH of the culture medium is an important factor affecting biosorption of heavy metals, and also that cation biosorption increases as solution pH increases (Arica et al. 2005; Gong et al. 2005; Gupta et al. 2006; Bayramoğlu and Arica 2008; King et al. 2008). Therefore, Zn removal by living cells of *S. obliquus* (L) was ascertained as a function of (initial) pH. The smallest extent of metal removal was recorded at the lowest pH tested, and the biosorption capacity increased with the increase in pH from 3 up to 6 (Fig. 5). The maximum biosorption capacity (112.8 mg Zn/g biomass) was accordingly achieved at pH 6, which corresponds to 78% of total metal initially present in the supernatant. One-way ANOVA was then performed on the data pertaining to Zn removal vs. pH. Among the pH values tested, the most efficient ones were 6 and 7, and the degrees of removal attained were not significantly ($P > 0.05$) different from each other; however, pH 6 led to removals that were

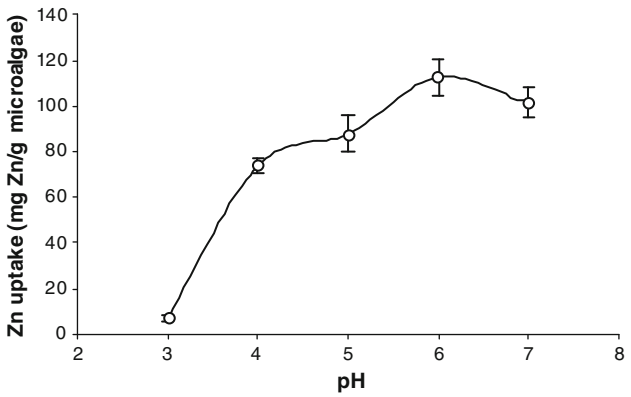

Fig. 5 Total amount of Zn removed, per unit biomass, by *Scenedesmus obliquus* (L), at 50 mg Zn/L, under several pH values. Results are expressed as means; *error bars* represent standard deviations ($n = 3$)

significantly ($P < 0.05$) higher than those at all other pH values tested, and removal at pH 3 was significantly ($P < 0.05$) lower than those at all remaining pH values.

The dependence of metal biosorption on pH is likely related to the ionization state of the functional groups on the surface of the microalgal cell walls (Arica et al. 2005; Bayramoğlu et al. 2006). At low pH, the cell surface sites are closely bound to H^+ ions, thereby making these unavailable for other cations (Gong et al. 2005; Bayramoğlu and Arica 2008); this accounts for the low biosorption capacity observed at pH 3. As pH increases, there is a concomitant increase in the number of ligands bearing negative charges (e.g. carboxylate groups) on the biomass surface, thus increasing attraction of metal ions and allowing biosorption onto the microalgal cell surface (Gong et al. 2005; King et al. 2008). Similar findings have been reported by Vannela and Verma (2006) pertaining to uptake of Zn, who found the maximum sorption capacity of *Spirulina platensis* at pH 6.0; by Bayramoğlu and Arica (2008), who found that the fungus *Lentinus edodes* reached the highest extent of metal removal also at pH 6.0; and by King et al. (2008), who observed an increase in Zn removal by *Azadirachta indica* when pH was increased, reaching its maximum at pH 6.

Therefore, metal removal by *S. obliquus* (L) cells depends strongly on the pH of the contaminated wastewaters; this microalgal biomass will perform better if removal is intended to take place at a pH value in the vicinity of neutrality 6.0–7.0.

Conclusions

The maximum extent of Zn removal (836.5 and 429.6 mg Zn/g biomass), by *S. obliquus* (L) and (ACOI), respectively, indicate that both microalga strains have a biosorption capacity comparable to other biosorbents. Nevertheless, the microalgal cells isolated from the polluted environment possess a higher tolerance and removal capacity than the strain obtained from the culture collection. Most Zn removal occurred via adsorption onto the cell surface, which follows the classical Langmuir isotherm pattern, and is consistent with previous knowledge on the tolerance mechanism of microalgae upon exposure to toxic metals in solution. Living cells outperform inactivated cells significantly, and the best removal is accomplished in the pH interval 6.0–7.0.

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